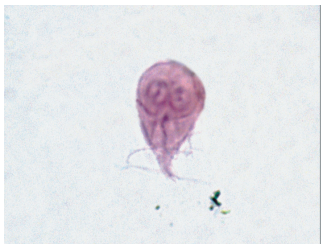

1

Introduction

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Introduction

Protozoa

Protozoa are single-celled eukaryotic animals first discovered by Antonie van Leeuwenhoek as he viewed *Giardia* in a personal enteric specimen through his own invention (above photos). For a summary of the phylogeny of protozoa, see Table 1.1. A recent trend is to replace the term "protozoa" with "protista." For these topics we retain "protozoa" and reserve "protista" for a much larger group of single-celled organisms that include the algae, other single-celled photosynthetic organisms, and some of the water molds (slime molds).

Note that *Pneumocystis jiroveci* (previously called *Pneumocystis carinii*) is not included in this volume.[1] Although this organism had been classified as a protozoon until the late 1980s, it is now considered a fungus.

Specimen Preparation

The diagnosis of most human parasitic infections relies upon the use of appropriate procedures for demonstrating the infecting organisms in feces, blood, urine, other body fluids and tissues. The most commonly applied procedures are briefly reviewed below. For an in-depth presentation of particular procedures, appropriate laboratory guides and atlases may be consulted. [2-4]

Stool

Fecal specimens may be preserved in 10% formalin, merthiolate-iodine-formalin (MTF) solution, sodium acetate-acetic acid-formalin (SAF) solution, Schaudinn fluid, or polyvinyl alcohol (PVA) combined with Schaudinn fluid. Schaudinn fluid contains mercury, hence it is banned in many laboratories. The quality of the morphology of organisms stained with trichrome or iron hematoxylin suffers when the modified fixative, in which zinc or copper is substituted for mercury, is used. Specimens should be first grossly examined to exclude macroscopic parasites such as roundworms or tapeworm proglottids. Concentration methods may be applied to fresh or preserved fecal specimens to detect light infections. The two most common procedures are zinc sulfate flotation and formalin-ethyl acetate sedimentation.

Wet mount preparations for microscopic examination may be prepared in normal saline or stained with iodine, methylene blue, trichrome or iron hematoxylin. Special

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stains may be used to demonstrate specific organisms, such as a modified acid-fast stain or modified safranin stain for *Cryptosporidium parvum* and *Cyclospora cayetanensis*. Ultraviolet fluorescence microscopy demonstrates the autofluorescence of coccidian cysts such as *C. cayetanensis* and *Isospora belli*. Calcofluor white may be used to brighten the fluorescence of coccidian oocysts. Kits for detecting antigens in feces are commercially available for several common parasites.

Important morphologic features of selected trophozoite forms are given in Table 1.2, and a summary of the comparative morphologic features of selected cyst forms is presented in Table 1.3.

Blood

Identification of most parasites in blood requires the preparation of stained thin or thick blood smears; however, specialized blood concentration techniques and commercial kits are available for the immunodetection of parasites, their antigens, and antibodies. Direct examination of drops of fresh blood or EDTA-preserved blood is useful for the detection of living motile trypomastigotes of *Trypanosoma* species. Preparation of thin or thick blood smears from fresh or EDTA-preserved blood followed by Giemsa, Wright or Dif-Quik® staining is useful for morphologic identification of species of *Plasmodium*, *Babesia*, *Trypanosoma* and *Leishmania*. For more details on blood smear preparation, see Topic 10. Specialized procedures exist for specific protozoans, such as buffy coat concentration for *Trypanosoma* and *Leishmania* species and cytocentrifugation concentration for *Plasmodium* and *Leishmania* species.[5]

Other fluids

Cytology specimens that may be examined for protozoa include urine, vaginal secretions, cerebrospinal fluid (CSF), aspirates of various tissues, and skin scrapings. Direct examination of the sediment of the first portion of voided urine may detect the motile flagellates of *Trichomonas vaginalis*, especially in male patients. Demonstration of *T. vaginalis* trophozoites in female patients is usually done by preparing wet mounts of vaginal swabs or scrapings. African trypanosomes and pathogenic free-living amebae (such as *Naegleria fowleri*) may be seen in CSF. Motile trypomastigotes of African trypanosomes can be found in aspirates from lymph nodes early in acute disease. Aspirates of bone marrow and spleen can demonstrate *Leishmania donovani*. Duodenal aspirates may be useful in demonstrating *Giardia lamblia*. Sigmoidoscopic material and aspirates of liver or lung abscesses may reveal trophozoites of *Entamoeba histolytica*. Cutaneous leishmaniasis can be diagnosed by finding amastigotes in stained smears prepared from scrapings of an ulcer.[6]

Tissue

The examination of tissue specimens for protozoan infections may be accomplished in several ways. Fresh, unfixed biopsy material can be used to make touch preparations, to inoculate culture media, or infect experimental animals. Fixed tissue can be examined as stained histologic specimens.

Arthopods & Pentastomes

Arthropods (phylum Arthropoda) are invertebrates with a chitinous exoskeleton, segmented body and jointed appendages. A summary of the phylogeny of arthropods is given in Table 1.4. This e-book covers diseases caused by arthropods that invade human tissue, including tungiasis (Topic 17), myiasis (Topic 18), and infestation with the non-toxic mites, *Sarcoptes* and *Demodex* species (Topic 19). Arthropods have many other significant relationships to human disease as vectors, or toxin-producers; however, those diseases are not addressed in this volume. The comparative morphologic characteristics of common parasitic arthropods is given in Table 1.5.

Pentastomes, or tongue worms, (Topic 16) are a class of parasitic animals that are similar to arthropods but that lack certain anatomic structures, such as circulatory and respiratory systems. The adults have oral hooks and the larvae have rudimentary legs; otherwise, they lack appendages.

Pseudoparasites and Artifacts

It is important to be able to distinguish artifacts, contaminants, non-pathogenic protozoa and other microorganisms from pathogenic protozoa. Structures that may mimic pathogenic protozoa vary with the specimen type.

Stool specimens contain a variety of objects that can be mistaken for protozoa, including plant material, especially pollen. Stool may also contain nonpathogenic protozoa, such as *Blastocystis hominis, Retortamonas intestinalis, Chilomastix mesnili, Enteromonas hominis, Endolimax nana, Iodamoeba buetschli, Entamoeba polecki, Entamoeba coli, Entamoeba hartmani* and *Entamoeba gingivalis*. Spores of *Myxobolus plectoplites*, a fish pathogen, have been reported in fecal samples from patients who had recently eaten fish.[7]

In blood films, artifacts, such as platelets superimposed on erythrocytes, staining artifacts, and an array of contaminants, may be mistaken for malarial parasites. Knowledge of the appearance of the normal constituents of blood prevents such errors.

Fluids from non-sterile body sites, such as sputum, are prone to contain foreign objects. Fluids from sterile body sites generally do not contain foreign objects, except in

cases where the fluid has become contaminated during collection or processing.

In tissue sections, one challenge is to distinguish minute protozoa from host cell structures. A common difficulty is discerning nuclear debris from amastigotes of *Leishmania*. Another problem may lie in deciding whether or not pigment deposition is due to *Plasmodium falciparum* infection. Yeast forms of some fungi, such as *Basidiobolus ranarum*, can bear a striking resemblance to amoebae. The intracytoplasmic inclusions of cytomegalovirus may be mistaken for intracellular microorganisms if one does not observe the diagnostic cytoplasmic inclusions of this virus.

Delusional parasitosis (Morgellons Disease)

Delusional parasitosis is a condition characterized by an isolated delusion by individuals that they are infested by parasites, especially ectoparasites or intestinal parasites. The patient, who usually has no other psychiatric conditions, often describes various symptoms of the skin or near body orifices. It is not uncommon that more than one family member has the same symptoms in a shared delusion (folie à deux). The typical patient collects specimens from skin, feces, clothing or environment. Parasitologists and pathologists are consulted to rule out the presence of actual parasites. The samples often consist of fibers, keratin, scabs, hairs, food particles or flies. Most patients see numerous health care providers and fiercely reject negative findings, and are reluctant to consult a psychiatrist. A dermatologist may be more successful at convincing the patient to begin appropriate psychotropic drugs or other therapy.[8,9]

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Table 1.1 Classification of Parasitic Protozoa. [a]

KINGDOM PROTOZOA

Subkingdom 1 Archezoa

Phylum Metamonada

Class Trepomonadea (intestinal flagellates)

Order Diplomonadida *Giardia lamblia* [b] (Topic 6)

Order Enteromonadida *Enteromonas hominis*

Class Retortamonadea

Order Retortamonadida *Chilomastix mesnili, Retortamonas intestinalis*

Phylum Parabasala (flagellates)

Class Trichomonadea (intestinal and related flagellates)

Order Trichomonadida *Trichomonas vaginalis*
T. tenax, Pentatrichomonas hominis (Topic 7)
Diantamoeba fragilis

Subkingdom 2 Neozoa

Infrakingdom 1 Discicristata

Phylum Percolozoa (flagellates)

Class Heterolobosea (flagellated amoebae)

Order Schizoprenida *Naegleria fowleri* (Topic 9)

Phylum Euglenozoa (flagellates)

Class Kinetoplastidea (kinetoplastid flagellates)

Order Trypanosomatida *Leishmania donovani, L. tropica* (Topic 4 & Topic 5)*; L. infantum* (Topic 5)*; L. major, L. braziliensis, L. mexicana, L. aethiopica, L. amazonensis, L. garnhami, L. guyanensis* (Topic 4); *L. colombiensis, L. lainsoni, L. naiffi, L. panamensis, L. peruviana, L. pifanoi, L. shawi;*
Trypanosoma cruzi, T. rangeli (Topic 2);
T. brucei gambiense, T. brucei rhodesiense (Topic 3)

Table 1.1 Classification of Parasitic Protozoa. [a] (Continued)

Infrakingdom 2 Sarcomastigota

Phylum Amoebozoa (amoebae)

Subphylum Lobosa

Class Amoebaea (amoeba)

Order Acanthopodida *Acanthamoeba castellanii, A. culbertsoni, A. hatchetti, A. polyphaga, Balamuthia mandrillaris* (Topic 9)

Subphylum Conosa

Class Entamoebidea (intestinal amoebae)

Order Euamoebida *Entamoeba histolitica, E. coli, E. dispar,E. hartmanni, E. gingivalis, E. moshkoviskii, E. polecki, Endolimax nana, Iodoamoeba bütschlii (Topic 8); E. chattoni*

Infrakingdom 3 Alveolata

Phylum Sporozoa (sporozoans)

Class Coccidea

Order Eimeriida *Cryptosporidium parvum, C. hominis, C. sp, Cyclospora cayetanensis, Isospora belli, Sarcocystis hominis, S. suihominis (*Topic 13*); S. lindemanni; Toxoplasma gondii* (Topic 12)

Order Piroplasmida *Babesia microti, B. divergens, B. gibsoni, Babesia sp* (Topic 11)

Order Haemosporida *Plasmodium falciparum, P. malariae, P. ovale, P. vivax (*Topic 10)

Phylum Ciliophora (ciliates)

Class Litostomatea

Order Vestibulifera *Balantidium coli* (Topic 15)

[a] Adapted from: Cox, FEG. Taxonomy and classification of human parasites. *Manual of Clinical Microbiology,* 9th edition, Volume 2, section X Parasitology, chapter 132, editor in chief PR Murray, editors EJ Barron, JH Jorgensen, ML Landry, MA Pfaller, volume editor MA Pfaller, section editor LS Garcia. Washington DC, ASM Press, 2007. p 1991.

Table 1.2 Morphologic Features of Selected Protozoan Trophozoite Forms

Trophozoites	Surface features	Shape	Size (μm)	Number of nuclei	Nuclear features	Other features
Balantidium coli	Cilia	Ovoid	50-200	1	Large macronucleus	Well-defined cytostome
Chilomastix mesnili	4 flagella, 3 anterior, 1 posterior	Round to pear-shaped	6-24	1	Large or small karyosome, evenly or irregularly distributed peripheral chromatin	Cytostome bordered by fibrils
Dientamoeba fragilis	Pseudopodia	Amoeboid	5-15	1 or 2	Karyosome fragmented into 4-8 pieces, no peripheral chromatin	Finely granular vacuolated cytoplasm, may contain bacteria, no erythrocytes
Endolimax nana	Pseudopodia	Amoeboid	6-12	1	Large karyosome, no peripheral chromatin	Coarsely granular cytoplasm, vacuolated, may contain bacteria, no erythrocytes
Entamoeba coli	Pseudopodia	Amoeboid	15-50	1	Large non-compact eccentric karyosome, coarse irregular peripheral chromatin	Coarsely granular cytoplasm, vacuolated, may contain bacteria, yeast or other debris, no erythrocytes
Entamoeba dispar	Pseudopodia	Amoeboid	20-60	1	Small compact usually central karyosome, fine evenly distributed peripheral chromatin	Finely granular cytoplasm, rarely contain erythrocytes
Entamoeba gingivalis	Pseudopodia	Amoeboid	20	1	Small central karyosome, fine granular regular peripheral chromatin	Finely granular cytoplasm, may contain bacteria, no erythrocytes, not found in feces
Entamoeba hartmanni	Pseudopodia	Amoeboid	5-12	1	Small compact usually central karyosome, fine evenly distributed peripheral chromatin	Finely granular cytoplasm, may contain bacteria, no erythrocytes
Entamoeba histolytica	Pseudopodia	Amoeboid	20-60	1	Small compact, usually central karyosome, fine evenly distributed peripheral chromatin	Finely granular cytoplasm, may contain erythrocytes

Table 1.2 Morphologic Features of Selected Protozoan Trophozoite Forms (Continued)

Trophozoites	Surface features	Shape	Size (μm)	Number of nuclei	Nuclear features	Other features
Entamoeba polecki	Pseudopodia	Amoeboid	10-25	1	Minute central karyosome, fine coarse or regular, irregular peripheral chromatin	Cytoplasm may contain bacteria or yeast, no erythrocytes
Enteromonas hominis	4 flagella, 3 anterior, 1 posterior	Round to oval	4-10	1	Large central karyosome	Flattened on one side
Giardia lamblia	4 pairs of flagella	Pear-shaped	10-20	2	Small central karyosome, no peripheral chromatin	Bilaterally symmetrical, median bodies lying transversely, two axonemes, no undulating membrane, ventral sucking disc
Iodamoeba bütschlii	Pseudopodia	Amoeboid	8-20	1	Large karyosome, achromic granules between karyosome and nuclear membrane, no peripheral chromatin	Coarsely granular cytoplasm, vacuolated, may contain bacteria, yeast or other debris, no erythrocytes
Pentatrichomonas hominis	5 flagella, 4 anterior, 1 posterior	Pear - shaped, oval	8-20	1	Karyosome usually central	Undulating membrane, axostyle, no median bodies
Retortamonas intestinalis	2 flagella, 1 anterior, 1 posterior	Pear-shaped or oval	4-10	1	Small karyosome, fine peripheral chromatin	Prominent cytostome

Table 1.3 Comparative Morphologic Features of Selected Protozoan Cyst Forms

Cysts	Type of cyst	Surface features	Shape	Size (μm)	Number of nuclei	Nuclear features	Other features
Balantidium coli	Not oocyst	Cilia within cyst wall	Round to oval	50-70	2	Large macro-nucleus, small micronucleus	Contractile vacuoles
Chilomastix mesnili	Not oocyst	No cilia	Round to lemon-shaped	6-10	1	Large, central karyosome	Hyaline knob or nipple-like protuberance, cytostome with fibrils
Cryptospo-ridium species	Sporulated oocyst	Modified acid-fast in stool only	Round	4-6	Not visible	–	4 naked sporozoites
Cyclospora cayetanensis	Unsporu-lated oocyst	Modified acid-fast, double cyst wall	Round	8-10	Not visible	–	2 sporocysts each with 2 sporozoites, greenish central mass, refractile globules
Endolimax nana	Not oocyst	No cilia	Round, oval or ellipsoidal	5-10	4	Large central karyosome, no peripheral chromatin	Small granules
Entamoeba coli	Not oocyst	No cilia	Round, oval, triangular	15-30	8 (mature), 1-4 (immature)	Large non-compact usually eccentric karyosome, coarse irregular peripheral chromatin more uniform than in trophozoites	Chromatoid bodies with splintered ends, central glycogen mass

Table 1.3 Comparative Morphologic Features of Selected Protozoan Cyst Forms (Continued)

Cysts	Type of cyst	Surface features	Shape	Size (μm)	Number of nuclei	Nuclear features	Other features
Entamoeba dispar	Not oocyst	No cilia	Round	10-20	4 (mature), 1-2 *(immature)*	Compact usually central small karyosome, fine evenly distributed peripheral chromatin,	Chromatoid bodies with rounded ends, central glycogen mass
Entamoeba hartmanni	Not oocyst	No cilia	Round	5-10	4 (mature), 1-2 *(immature)*	Small compact usually central karyosome, fine evenly distributed peripheral chromatin	Grape-like chromatoid bodies with bluntly rounded ends
Entamoeba histolytica	Not oocyst	No cilia	Round	10-20	4 (mature), 1-2 *(immature)*	Small compact usually central karyosome, fine evenly distributed peripheral chromatin	Chromatoid bodies with rounded ends, central glycogen mass
Entamoeba polecki	Not oocyst	No cilia	Round, oval	10-15	1	Small central or eccentric karyosome, fine regular peripheral chromatin	Many grape-like chromatoid bodies or dark-staining inclusion mass
Enteromonas hominis	Not oocyst	No cilia	Oval or ellipsoidal	4-8	2-4	1 or 2 nuclei at each end, small central karyosome, no peripheral chromatin	Intracytoplasmic flagella
Giardia lamblia	Not oocyst	No cilia	Ovoid to ellipsoidal	8-19	4 (mature) 2 (immature)	4 nuclei at one end central karyosome, no peripheral chromatin	Axonemes, intracytoplasmic fibrils lying transversely

Table 1.3 Comparative Morphologic Features of Selected Protozoan Cyst Forms (Continued)

Cysts	Type of cyst	Surface features	Shape	Size (μm)	Number of nuclei	Nuclear features	Other features
Iodamoeba buetschlii	Not oocyst	No cilia	Oval, ellipsoidal, triangular	5-20	1	Large eccentric karysome, no peripheral chromatin	Large glycogen vacuole
Isospora belli	Unsporulated oocyst contains one immature sporont	Modified acid-*fast*	Elongated	20-33	Not visible	–	Mature oocyst contains 2 sporocysts each with 4 sporozoites
E. bieneusi Microsporidia	Spore	Modified Trichrome stain	Oval	1-5	Not visible	-	
Retortamonas intestinalis	Not oocyst	No cilia	Pear-shaped or oval	4-9	1	Slightly eccentric, small compact karyosome, variable peripheral chromatin	Fibril and cytostome near nucleus
Sarcocystis species	Sporulated oocyst	Thin-walled	Elongated	15-19	Not visible	–	2 sporocysts each with 4 sporozoites, single sporozoites from ruptured oocysts

TABLE 1.4 CLASSIFICATION OF PATHOGENIC ARTHROPODS AND PENTASTOMES

1. Phylum Arthropoda

   a. Subphylum mandibulata
      Class Insecta (fleas, flies, mosquitoes, bees, lice, bugs, beetles, caterpillars, fire ants)

   b. Subphylum Chelicerata
      Class Arachnida (spiders, scorpions, mites, ticks)

   c. Subphylum Onychophora (centipedes, millipedes)

2. Phylum Pentastomida

      Class Pentastomata

Table 1.5 Comparative Morphologic Features of Selected Arthropods in Histologic Sections

Organism	Anatomic location	Cuticle		Hypodermis	Eggs (μm)	Legs
		Thickness (μm)	Features			
Tick	Above keratin	>50	Long, thin spurs, striated	Thin	No	Yes
Sarcoptes scabiei	Stratum corneum	<5	Sharply pointed spines, striated	Very thin	Yes 150	Yes
Tunga penetrans	Stratum corneum	>50	No spines	Thick	Yes 600	Yes
Dermatobia hominis	Dermis, Subcutis	>50	Spines in rows	Thin	No	No
Cordylobia anthropophagia	Dermis, Subcutis	>25	Spines smaller, sparse, irreguarly distributed	Thin	No	No